while Y639F is also capable of unprimed reverse transcription.

DNA- and RNA-primed synthesis of DNA and RNA: In the assays described so far we have examined *de novo* initiated synthesis. T7 RNAP can also extend RNA primers. We, therefore, examined the abilities of both polymerase to carry out DNA or RNA primed synthesis of DNA and RNA (Fig. 5).

Fig. 5 shows primed synthesis of DNA and RNA with Y639F and the w.t. polymerase. Transcription reactions contained end-labeled 12 base DNA or RNA primers of identical sequence (GGACACGGCGAA, SEQ ID NO: 4) hybridized to a DNA template (CCCGGGATGGAATGGAGTATTCGCCGTGTCCATGGCTGTAAGTATCC, SEQ ID NO: 5). Primer-template concentration was 10^{-5} M. Reactions contained the indicated polymerases (10^{-7} M) and NTPs. NTP concentrations and electrophoresis as in Fig. 1.

We found that both the w.t. and Y639F polymerases can extend DNA and RNA primers with rNTPs, but extension of DNA primers was 2-3-fold less efficient than extension of RNA primers. Y639F also extended DNA and RNA primers with dNTPs but ~4-fold less efficiently than with rNTPs.

The Y639F mutant does not exhibit greatly increased miscoding: We examined the miscoding properties of the w.t. and mutant T7 RNAPs by measuring the relative incorporation of labeled rGTP, rUTP, rATP, and rCTP on poly(dC) or poly(dT) templates in the presence of excess unlabeled rGTP or rATP, respectively (Table V, see Appendix 1). An increase in miscoding would be reflected in an increase in the rate of incorporation of the non-complementary NTP into RNA.

On poly(dC), the w.t., Y639F, and G640A polymerases incorporate rGTP into RNA at greater than 1300-2000-fold the

rate of rUTP incorporation. Ribo-GTP is incorporated some 400-600- fold better than rCTP, and 200-400-fold better than rATP. Because of their lower activity we can say only that the relative rate of incorporation of rGTP on poly(dC) is 184-fold greater than the rate of incorporation of non-complementary rNTPs for Y639A, and 50-fold greater for Y639S. The use of Mn^{++} instead of Mg^{++} has been reported to increase miscoding for a number of polymerases (Tabor and Richardson, 1989; Nivogi and Feldman, 1981) so we examined the effects of Mn^{++} on miscoding by w.t. and Y639F polymerases. In Mn^{++} buffer the G640A, Y639A, and Y639S mutants were insufficiently active to allow accurate measures of miscoding. With the w.t. and Y639F polymerases the use of Mn^{++} increases miscoding by 20-40-fold. However, the apparent rate of miscoding by Y639F remains similar to the w.t enzyme.

On poly(dT), high levels of activity allowing an accurate measure of miscoding frequencies could only be observed for Y639F and the w.t. polymerase in both Mg^{++} and Mn^{++} buffers. In Mg^{++} buffer apparent miscoding rates on poly(dT) were higher than on poly(dC), but were similar for Y639F and the w.t. enzyme. In Mn^{++} buffer miscoding rates were increased by ~5-fold, on average, but again rates were similar for w.t. and Y639F. However, on poly(dT) Y639F did show a reproducible ~2-fold increase, relative to the w.t. enzyme, in the ratio of the rates of rGTP to rATP incorporation.

Homopolymer assays have been used previously to measure miscoding by RNAPs (Nivogi and Feldman, 1981; Glazer, 1978; Blank, et al., 1986) but it should be remarked that they can produce only upper bounds for miscoding frequencies. Measured miscoding rates could reflect contamination of the

homopolymeric templates. It is also possible that the transcripts themselves could serve as templates and support incorporation of rNMPs non-complementary to the original template (i.e., the poly(rG) or poly(rA) transcripts made on poly(dC) or poly(dT) could subsequently support synthesis of poly(rC) or poly(rU) transcripts). Such caveats are less relevant to the miscoding observed in Mn^{++} buffer since the change in divalent cation increases miscoding but cannot affect template composition. However, in Mg^{++} buffer we should consider the measured miscoding frequencies to be upper bounds for the true rates of miscoding. Nevertheless, the results presented in table V indicate that Y639F does not exhibit a gross increase in miscoding which would manifest itself as a clear increase in the incorporation of non-complementary rNMPs on homopolymeric templates.

The increased utilization of dNTPs by Y639F is due to both a decreased K_m and an increased k_{cat} for dNTPs: The K_m and k_{cat} of the w.t. and Y639F polymerases with rATP, rITP and 5 different dNTPs were measured (Table VI, see Appendix 1). The K_m of the w.t. enzyme for dNTPs was much higher than previously reported values for the corresponding rNTPs and varied considerably for different dNTPs (Ikeda and Richardson, 1987; Patra, et al., 1992). Notably the w.t. enzyme K_m values correlate with the rNTP/dNTP selectivity values presented in Table I. The selectivity of the w.t. enzyme with ribo- vs. deoxy-nucleotides was greatest for CTP, followed by ATP, and was the least for UTP, implying that an important component of the selectivity of the w.t. enzyme for rNTPs over dNTPs is a much higher K_m for dNTPs. For Y639F, the K_m values for these dNTPs are from ~3 to ~11-fold less, but the rank order of these K_m values (dCTP K_m> dATP K_m> dGTP K_m> dTTP K_m) is the same as for the w.t.